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# Autoradiographic localization of 5-$HT_1$ receptors to human and canine basilar arteries

STEPHEN J. PEROUTKA[1] and MICHAEL J. KUHAR

*Departments of [1]Neurology and Neuroscience, Meyer 1-190, The Johns Hopkins Hospital, Baltimore, MD 21205 (U.S.A.)*

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Autoradiographic techniques were used to identify serotonergic binding sites in human and canine basilar artery segments. 5-$HT_1$, but not 5-$HT_2$, receptors could be localized to the medial layer of the vascular wall using both [$^3$H]LSD and [$^3$H]5-HT. The identification of 5-$HT_1$ binding sites in human and canine basilar arteries provides anatomical evidence that the 5-$HT_1$ receptor mediates vasoconstriction in these major intracranial vessels.

Serotonin (5-hydroxytryptamine; 5-HT) has been implicated in the pathogenesis of vascular disorders such as migraine[26] and cerebral ischemia following subarachnoid hemorrhage[2,27,29]. In non-human species, serotonergic axonal networks have been demonstrated in both the major intracranial vessels and the cerebral microcirculation[4,5,8–11,14,20,24,25]. Physiologically, 5-HT is a potent vasoconstrictor of human basilar, middle cerebral and pial vessels[1,12,13,15–17]. However, the effects of serotonergic agonists and antagonists vary markedly depending on the specific vessel under study. Recently, the interactions of serotonergic agents with vascular smooth muscle have been correlated with 5-$HT_1$[22,28] and 5-$HT_2$[6,7,18] receptor subtypes as defined by radioligand binding studies in brain membranes[23]. Since 5-$HT_1$ and 5-$HT_2$ subtypes of serotonergic receptors have markedly different affinities for a variety of agonist and antagonist drugs, the identification of a specific receptor subtype in intracranial arteries may help guide the selection of agents used in the treatment of cerebrovascular disorders. In the present study, we report the direct identification of 5-$HT_1$, but not 5-$HT_2$, receptors in human and canine basilar arteries by receptor binding autoradiographic techniques.

In order to detect possible binding to 5-$HT_1$ and/or 5-$HT_2$ receptors, serial mounted sections of human and canine basilar arteries were incubated with 8 nM [$^3$H]LSD and the slides were processed for autoradiography[30]. In the absence of inhibitors, specific grain density was 10 ± 0.9 grains/625 $\mu m^2$ ($P < 0.001$) in human and 21 ± 4 grains/625 $\mu m^2$ ($P < 0.02$) in canine basilar arteries (Table I). This amount of specific binding represented 33% and 43% of total binding observed in human and canine vessels, respectively. When [$^3$H]LSD was incubated with 300 nM 5-HT in order to restrict radioligand binding to 5-$HT_2$ receptors, as defined in brain tissues, specific grain density was significantly reduced to 2 ± 0.5 grains/625 $\mu m^2$ in human and 4 ± 2 grains/625 $\mu m^2$ in canine arteries. These values were not significantly greater than non-specific binding (defined as [$^3$H]LSD grain density observed in the presence of 300 nM 5-HT + 30 nM spiperone). In the presence of 30 nM spiperone, [$^3$H]LSD selectively labels 5-$HT_1$ receptors[23]. Under these conditions, significant amounts of specific binding could be detected in human (10 ± 0.8 grains/625 $\mu m^2$; $P < 0.001$) and canine (22 ± 2 grains/625 $\mu m^2$; $P < 0.02$) basilar artery segments. No significant difference ($P > 0.05$) was observed between the specific grain density in the presence of 300 nM 5-HT and the specific grain

*Correspondence:* S. J. Peroutka, Departments of Neurology and Pharmacology, Stanford University Medical Center, Stanford, CA 94305, U.S.A.

TABLE I

*Binding of [$^3$H]ligands to 5-HT receptors in human and canine basilar artery*

The binding of 8 nM [$^3$H]LSD and 4 nM [$^3$H]5-HT was analyzed in the absence or presence of inhibitor drugs. Grain density measurements represent the average number of grains counted in 4 regions (625 $\mu m^2$) of a single section of artery using 40 × magnification. Data given are the average number ± standard error of specific grain counts/625 $\mu m^2$ taken from 3 arteries, each of which was evaluated in 4–7 different sections. Specific grain counts were defined as the excess number of grains/section over blanks taken in the presence of 300 nM 5-HT + 30 nM spiperone for [$^3$H]LSD binding and 300 nM 5-HT for [$^3$H]5-HT binding. The standard *t*-test was used for statistical comparisons of mean values.

| *[$^3$H]Ligand* | *Displacing agent* | *Receptor subtype specifically labeled* | *Human grain density (counts/region)* | *Canine grain density (counts/region)* |
|---|---|---|---|---|
| [$^3$H]LSD | – | 5-$HT_1$ + 5-$HT_2$ | 10 ± 0.9** | 21 ± 4* |
| [$^3$H]LSD | 300 nM 5-HT | 5-$HT_2$ | 2 ± 0.5 | 4 ± 2 |
| [$^3$H]LSD | 30 nM spiperone | 5-$HT_1$ | 10 ± 0.8** | 22 ± 2* |
| [$^3$H]5-HT | – | 5-$HT_1$ | 24 ± 3** | 14 ± 4* |

* $P < 0.02$; ** $P < 0.001$ in comparison to non-specific grain density values.

density in the presence of 300 nM 5-HT + 30 nM spiperone.

Because the specific binding of [$^3$H]LSD suggested the labeling of 5-$HT_1$ but not 5-$HT_2$ receptors, we attempted to confirm the presence of 5-$HT_1$ receptor sites with the binding of [$^3$H]5-HT. Specific grain density using 4 nM [$^3$H]5-HT was defined as the excess over blanks taken in the presence of 300 nM 5-HT and represented 37% and 33% of binding observed in human and canine arteries, respectively. Significant amounts of specific grain counts were observed in both human (24 ± 3 grains/625 $\mu m^2$; $P < 0.001$) and canine (14 ± 4 grains/625 $\mu m^2$; $P < 0.02$) basilar artery segments (Table I). As seen in Fig. 1, the labeled sites were homogeneously located throughout the medial layer (tunica media) of the blood vessel.

The major finding of the present study is that 5-$HT_1$, but not 5-$HT_2$, receptors can be identified on intracranial vascular smooth muscle. The differentiation of 5-$HT_1$ and 5-$HT_2$ receptors was based on the distinctive pharmacological characteristics of membrane binding sites identified in brain homogenates[23]. Although the physiologic interactions of serotonergic agents with the canine basilar artery suggests an association with 5-$HT_1$ receptors[22,28], radioligand filtration studies are not sensitive enough to identify binding sites in small, difficult to obtain, muscular tissues. The autoradiographic localization of 5-$HT_1$ receptors to human and canine basilar artery provides anatomical evidence that the 5-$HT_1$ receptor mediates contraction in these blood vessels.

Innervation of intracranial blood vessels by 5-HT has been observed with a variety of techniques. Chan-Palay[4,5] demonstrated serotonergic plexuses around major cerebral and pial vessels in the rat and monkey. In the rabbit vertebral artery, a 5-HT immunoreactive nerve plexus and the 5-HT metabolite 5-hydroxyindoleacetic acid have been demonstrated[14]. Using a peroxidase-antiperoxidase histochemical technique utilizing antibodies specific for 5-HT, DiCarlo and Beitz[10] have shown prominent axonal networks in the adventitia of the rat basilar artery and branches of the carotid circulation. Reinhard et al.[24,25] found 5-HT in purified samples of microvessels from rat and canine brain. Electrolytic and pharmacologic lesions of the central raphe nuclei decreased the vessel content of 5-HT suggesting a direct innervation of small intrinsic vessels by serotonergic neurons. These findings have recently been confirmed in rat pial vessels[11]. As a result, intracranial vasculature appears to be directly innervated by brainstem 5-HT nuclei.

The effect of 5-HT on vascular smooth muscle, however, varies markedly depending on the specific vessel under study. The significant differences between agonist and antagonist potencies in different vascular systems have led many investigators to propose the existence of multiple vascular 5-HT receptors[3,13,16,19,21]. In general, two distinct responses to 5-HT have been observed using in vitro contraction techniques. First, certain vessels have high affinity

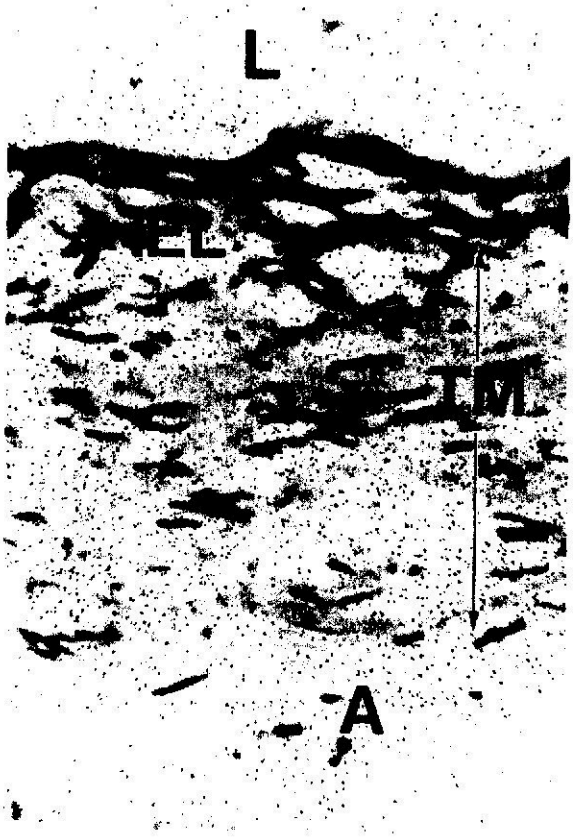


Fig. 1. Autoradiographic identification of [$^3$H]5-HT binding sites in human basilar artery. Shown is an autoradiogram of a human basilar artery labeled with [$^3$H]5-HT. The photograph was taken at 40 × magnification with the autoradiographic grains in focus. Grain density is highest over the tunica media (TM) and internal elastic lamina (IEL). Fewer grains are located over the adventitia (A). The lumen (L) of the artery is essentially devoid of radiographic grains. Human basilar arteries were obtained at autopsy and canine basilar arteries were obtained from adult dogs after death by rapid exsanguination. The arteries were dissected into 4 mm segments and placed in embedding matrix on a cryostat chuck. The assembly was frozen in liquid freon. Sections (6 $\mu$m) were cut on a cryostat microtome and thaw-mounted onto subbed slides. 5-HT receptors were labeled by incubation of the slide-mounted sections in 8 nM [$^3$H]LSD (New England Nuclear, Boston, MA; 56.2 Ci/mmol) or 4 nM [$^3$H]5-HT (New England Nuclear, Boston, MA; 33 Ci/mmol) for 60 min at 25 °C in 50 mM Tris-HCl buffer (pH 7.6) and 4 mM calcium chloride. For [$^3$H]LSD sites, 300 nM 5-HT and/or 30 nM spiperone were used to evaluate specific binding to 5-HT$_1$ or 5-HT$_2$ receptors. For [$^3$H]5-HT studies, blanks were generated by adding 300 nM 5-HT to the incubation solution. Following three consecutive 5-min washes in cold buffer without drugs, the mounted sections were dried and processed for autoradiography as previously described[30]. The exposure duration was 9 weeks.

for 5-HT but the contraction is essentially resistant to blockade by classical antagonists such as methysergide, cyproheptadine and cinanserin. A second response has been observed in which the vessel has comparatively low affinity for 5-HT but is completely blocked by low concentrations of the classical antagonists. These two distinct responses may correlate with the 5-HT$_1$ and 5-HT$_2$ receptors, respectively, that have been described in brain membranes[21]. The present report represents the first direct demonstration of 5-HT$_1$ receptor sites in vasculature. The receptors are homogeneously located over the entire vascular wall. In the canine preparation, these data confirm the conclusion (drawn from physiologic studies) that contraction of the canine basilar artery is mediated via 5-HT$_1$ receptors[22,28].

In the human, identification of 5-HT$_1$ but not 5-HT$_2$ receptors in a major intracranial vessel may have important clinical implications. Diseases such as migraine and vasospasm following subarachnoid hemorrhage are thought to occur as a result of abnormal vasoconstriction. Since 5-HT is a potent endogenous vasoconstrictor of intracranial vasculature, a selective pharmacologic blockade of 5-HT$_1$ receptors might be beneficial in certain clinical situations. However, to date, a specific 5-HT$_1$ antagonist has not been described. Development of such an agent may have useful clinical applications in the treatment of vascular neurologic diseases such as migraine, vasospasm and ischemia.

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1 Allen, G. S., Gross, C. J., French, L. A. and Chou, S. N., Cerebral Arterial Spasm. Part 5: In vitro contractile activity of vasoactive agents including human cerebrospinal fluid on human basilar and anterior cerebral arteries, *J. Neurosurg.*, 44 (1976) 594–600.

2 Allen, G. S., Henderson, L. M., Chou, S. N. and French, L. A., Cerebral Arterial Spasm. Part 1: In vitro contractile activity of vasoactive agents on canine basilar and middle cerebral arteries, *J. Neurosurg.*, 40 (1974) 433–441.

3 Apperley, E., Feniuk, W., Humphrey, P. P. A. and Levy, G. P., Evidence for two types of excitatory receptor for 5-hydroxytryptamine in dog isolated vasculature, *Brit. J. Pharmacol.*, 68 (1980) 215–224.

4 Chan-Palay, V., Serotonin axons in the supra- and subependymal plexuses and in the leptomeninges; their roles in local alterations of cerebrospinal fluid and vasomotor activity, *Brain Research*, 102 (1976) 103–130.

5 Chan-Palay, V., Innervation of cerebral blood vessels by norepinephrine, indoleamine, substance P and neurotensin fibers and the leptomeningeal indoleamine axons: their roles in vasomotor activity and local alterations of brain blood composition. In *Neurogenic Control of the Brain Circulation*, Pergamon Press, Oxford, 1977, pp. 39–53.

6 Cohen, M. L., Fuller, R. W. and Wiley, K. S., Evidence of 5-$HT_2$ receptors mediating contraction in vascular smooth muscle, *J. Pharmacol. exp. Ther.*, 218 (1981) 421–425.

7 Cohen, M. L., Mason, N., Wiley, K. S. and Fuller, R. W., Further evidence that vascular serotonin receptors are of the 5-$HT_2$ type, *Biochem. Pharmacol.*, 32 (1983) 567–570.

8 DiCarlo, V., Serotoninergic innervation of extrinsic brainstem blood vessels. In *Neurogenic Control of the Brain Circulation*, Pergamon Press, Oxford, 1977, pp. 55–58.

9 DiCarlo, V., Histochemical evidence for a serotonergic innervation of the microcirculation in the brainstem, *Neurology*, 31 (1981) 104.

10 DiCarlo, V. and Beitz, A. J., Immunohistochemical evidence for serotoninergic innervation of brainstem blood vessels, *Neurology*, 33 (1983) 73.

11 Edvinsson, L., Degueurce, A., Duverger, D., MacKenzie, E. T. and Scatton, B., Central serotonergic nerves project to the pial vessels of the brain, *Nature (Lond.)*, 306 (1983) 55–57.

12 Edvinsson, L., Hardebo, J. E. and Owman, Ch., Pharmacological analysis of 5-hydroxytryptamine receptors in isolated intracranial and extracranial vessels of cat and man, *Circulat. Res.*, 42 (1978) 143–151.

13 Forster, C. and Whalley, E. T., Analysis of the 5-hydroxytryptamine induced contraction of the human basilar arterial strip compared with the rat aortic strip in vitro, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 319 (1982) 12–17.

14 Griffith, S. G. and Burnstock, G., Serotonin as a neurotransmitter in cerebral arteries, *Brain Research*, 247 (1982) 388–392.

15 Hardebo, J. E., Edvinsson, L., Owman, Ch. and Svendgaard, N.-Aa., Potentiation and antagonism of serotonin effects on intracranial and extracranial vessels, *Neurology*, 28 (1978) 64–70.

16 Lamar, J. C. and Edvinsson, L., 5-Hydroxytryptamine receptors. Contractile activity and mode of inhibition by methysergide in mammalian intracranial and extracranial vessels, *Arch. int. Pharmacodyn.*, 243 (1980) 245–254.

17 Lamar, J. C. and Hardebo, J. E., Interaction of a potential antimigraine drug (ORG GC 94) with the vasomotor action of serotonin, *Europ. J. Pharmacol.*, 60 (1979) 263–275.

18 Leysen, J. E., Niemegeers, C. J. E., Van Nueten, J. M. and Laduron, P. M., $^3$H-Ketanserin (R 41 468), a selective $^3$H-ligand for serotonin$_2$ receptor binding sites, *Molec. Pharmacol.*, 21 (1982) 301–314.

19 Muller-Schweinitzer, E., The mechanism of ergometrine-induced coronary arterial spasm: in vitro studies on canine arteries, *J. Cardiovasc. Pharmacol.*, 2 (1980) 645–655.

20 Napoleone, P., Sancesario, G. and Amenta, F., 5-Hydroxytryptophan uptake in indoleaminergic nerve fibers within rat cerebrovascular tree, *Neurosci. Lett.*, 28 (1982) 57–60.

21 Peroutka, S. J., Vascular serotonin receptors: correlation with 5-$HT_1$ and 5-$HT_2$ binding sites, *Biochem. Pharmacol.*, (1984) in press.

22 Peroutka, S. J., Noguchi, M., Tolner, D. J. and Allen, G. S., Serotonin-induced contraction of canine basilar artery: mediation by 5-$HT_1$ receptors, *Brain Research*, 259 (1983) 327–330.

23 Peroutka, S. J. and Snyder, S. H., Multiple serotonin receptors: differential binding of $^3$H-serotonin, $^3$H-lysergic acid diethylamide and $^3$H-spiroperidol, *Molec. Pharmacol.*, 16 (1979) 687–699.

24 Reinhard, Jr., J. F., Liebmann, J. E., Schlosberg, A. J. and Moskowitz, M. A., Serotonin neurons project to small blood vessels in the brain, *Science*, 206 (1979) 85–87.

25 Reinhard, Jr., J. F., Moskowitz, M. A., Elspas, S. R. and Liebmann, J. E., Serotoninergic innervation of rat and bovine parenchymal brain blood vessels: biochemical and pharmacological studies, *Soc. Neurosci. Abstr.*, 4 (1978) 451.

26 Sicuteri, F., Vasoneuroactive substances and their implication in vascular pain, *Res. Clin. Stud. Headache*, 1 (1967) 6–45.

27 Svendgaard, N.-Aa., Edvinsson, L., Owman, Ch. and Sahlin, Ch., Increased sensitivity of the basilar artery to norepinephrine and 5-hydroxytryptamine following experimental subarachnoid hemorrhage, *Surg. Neurol.*, 8 (1977) 191–195.

28 Taylor, E. W., Duckles, S. P. and Nelson, D. L., Vascular preparations: use in the development of serotonergic agents specific for receptor subtypes, *Soc. Neurosci. Abstr.*, 9 (1983) 335.

29 Yashon, D., Blocker, D., Brown, R. J. and Hunt, W. E., Quantitative in vitro vasoactivity of 5-hydroxytryptamine on the human basilar artery, *Surg. Neurol.*, 3 (1975) 295–300.

30 Young, III, W. S. and Kuhar, M. J., Serotonin receptor localization in rat brain by light microscopic autoradiography, *Europ. J. Pharmacol.*, 62 (1980) 237–239.